Technical Explanation

US Application 10/583,706

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Claim 1 of the present application

(TP-FP primer set)

A primer set comprising at least two primers that allows a target nucleic acid sequence to be amplified,

wherein a **first primer** included in the primer set contains, in its 3' end portion, a sequence (Ac') that hybridizes to a sequence (A) located in the 3' end portion of the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Ac'), a sequence (B') that hybridizes to a complementary sequence (Bc) to a sequence (B) that is present on the 5' side with respect to the sequence (A) in the target nucleic acid sequence, and

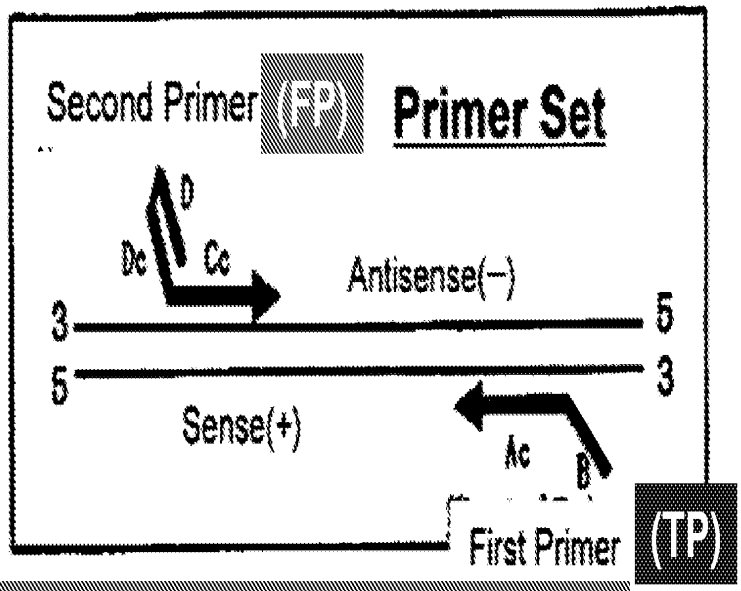
a **second primer** included in the primer set contains, in its 3' end portion, a sequence (Cc') that hybridizes to a sequence (C) located in the 3' end portion of a complementary sequence to the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Cc'), a folded sequence (D-Dc') that contains, on the same strand, two nucleic acid sequences that hybridize to each other.

*The first primer is **TP**, the second primer is **FP**.

TP; Turn-back Primer

FP; Folded Primer

Technical explanation of the TP and FP



FP functions as follows:

(1) FP has the folded sequence (D-Dc') in the 5' side sequence.

(2) The folded sequence (D-Dc') has two nucleic acid sequences that hybridize to each other.

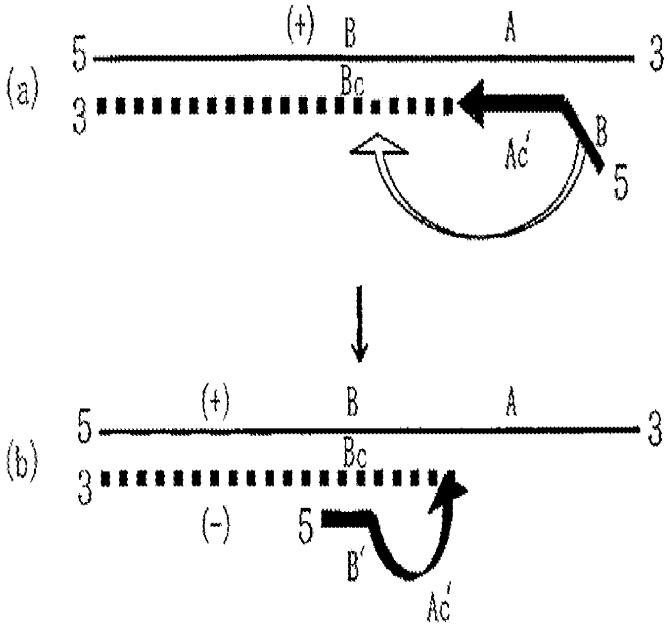
(3) The folded sequence (D-Dc') **DOES NOT** hybridize to the elongation strand from FP (**NO turn back**).

(4) **NO** primer hybridizes to the folded sequence (D-Dc') of the FP or the elongation strand from FP.

TP functions as follows:

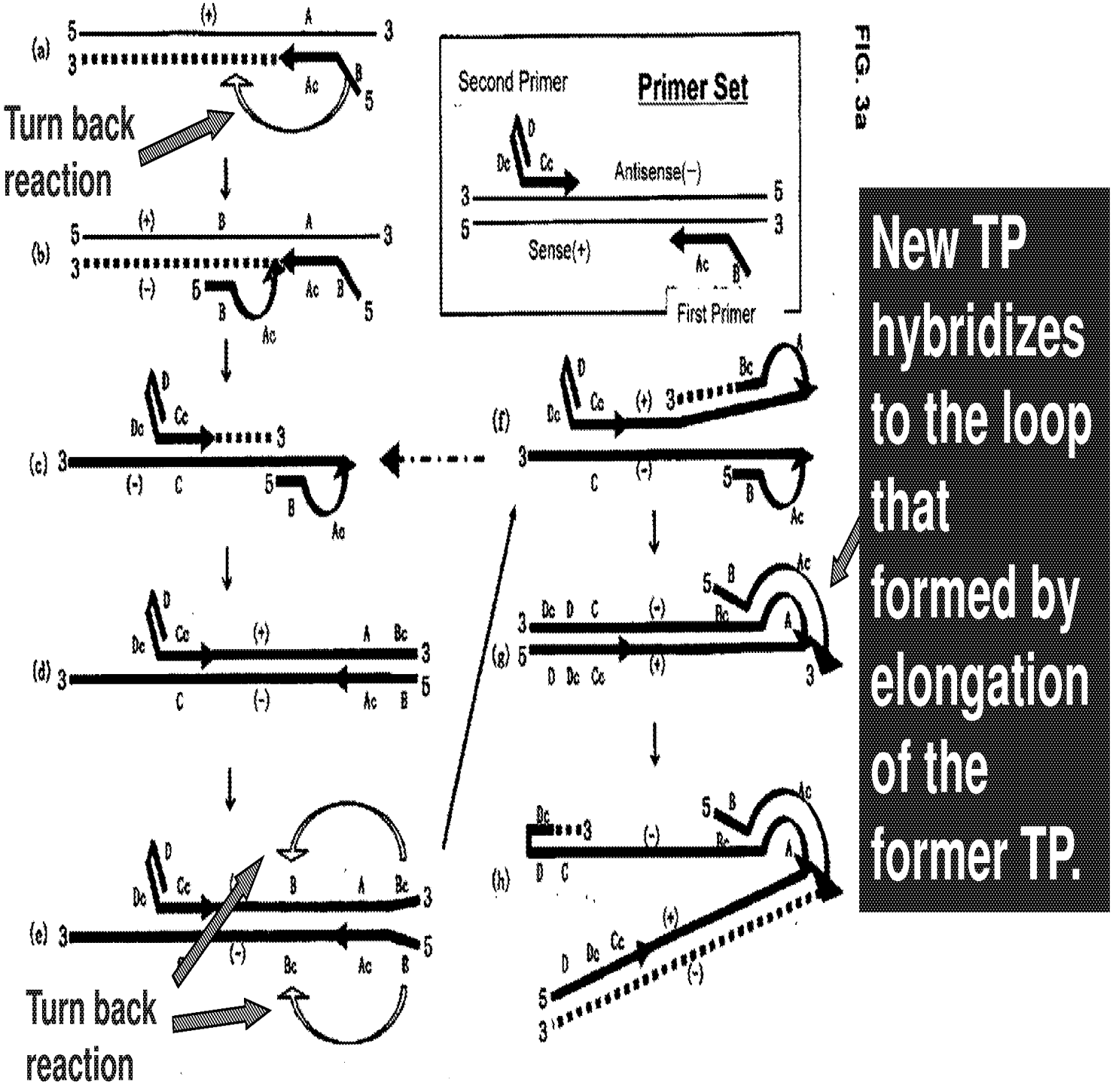
(1) TP has the turn back portion (B) in the 5' side sequence.

(2) The turn back portion (B) can hybridize to the portion (Bc) of the elongation strand from TP.



Mechanism of the amplification reaction of the TP-FP(1)

(FIG.3 of the present invention)



Mechanism of the amplification reaction of the TP-FP(2)

(FIG.3 of the present invention)

The characteristic feature of the FP itself or the folded portion formed by elongation of the former FP is that these portions do NOT hybridize to any primers. This feature enables us to prevent background amplification.

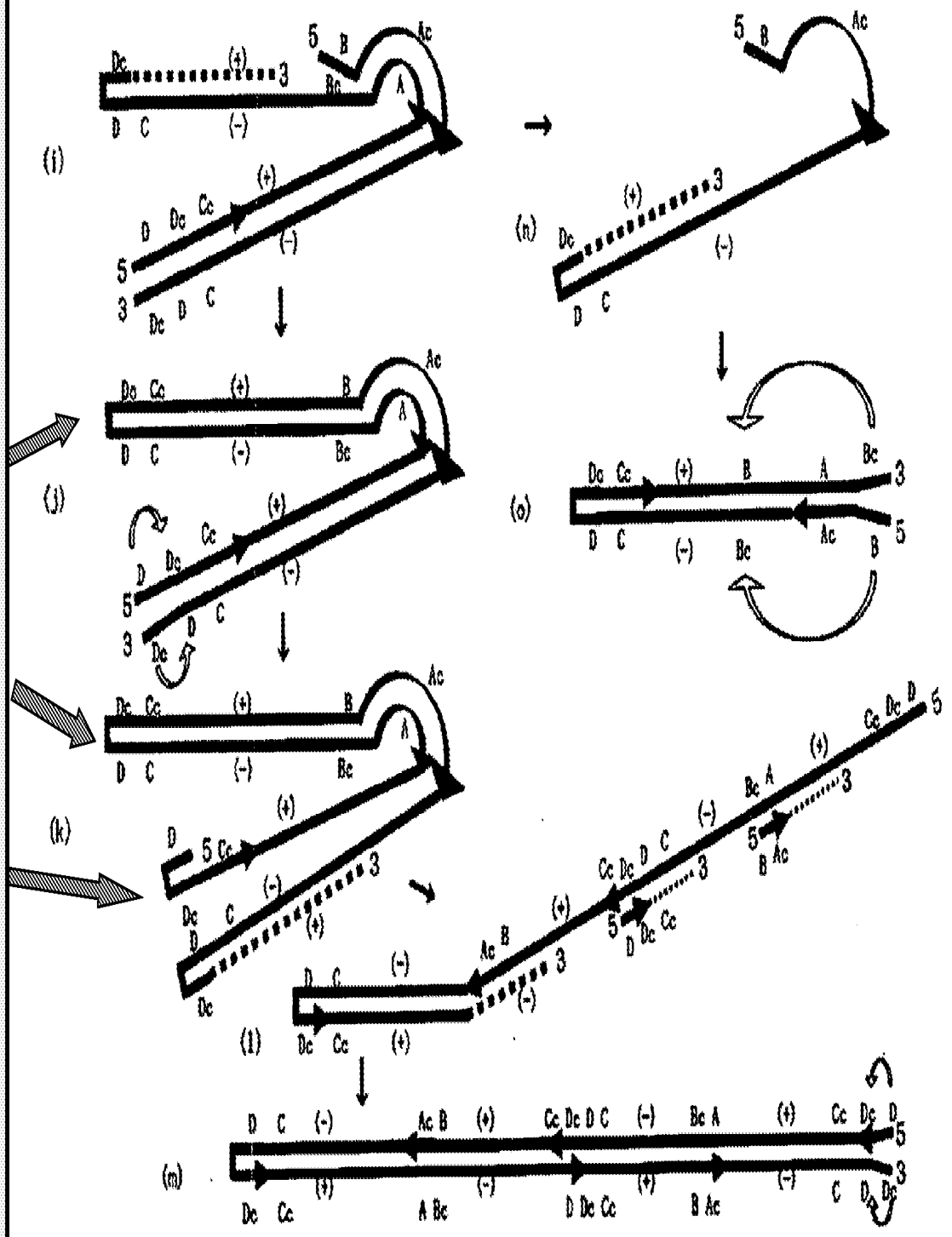


FIG. 3b

Summary of the Office Action

The Office Action explained the following:

(1) TP are shown in Figure 4, step 1 and 2 (① and ② shown in below) in Rabbani (EP0971039A2).

(2) Rabbani teaches that second primer can be different from initial primer (paragraph 43, p. 8).

(3) FP are shown in Fig. 5b in David (WO96/001327).

(4) A portion CB'C' in Figure 4 ① in Rabbani matches quite well with FP (Response to Arguments).

(5) Therefore, Claims 1 to 7 of the present invention are obvious (103(a)).

FP-like portion pointed out by the examiner.

TP pointed out by the examiner.

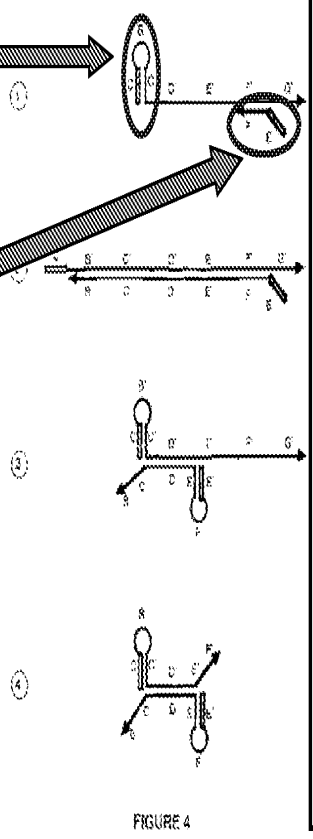


FIGURE 4

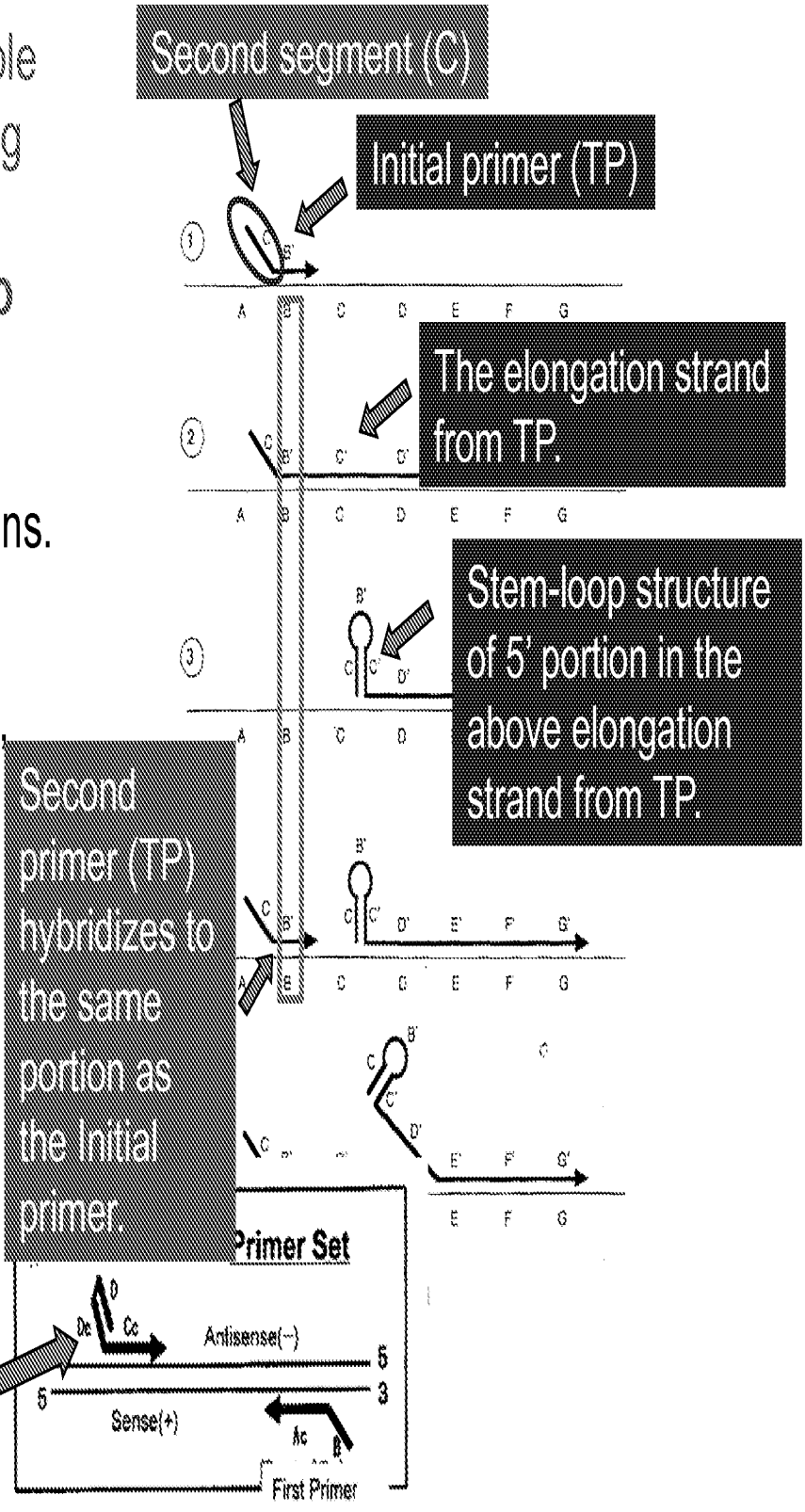
With regard to (2), second primer is NOT reverse primer (FP).

With regard to (4), the portion CB'C' in Figure 4 ① in Rabbani does NOT match folded sequence (D-Dc') of FP.

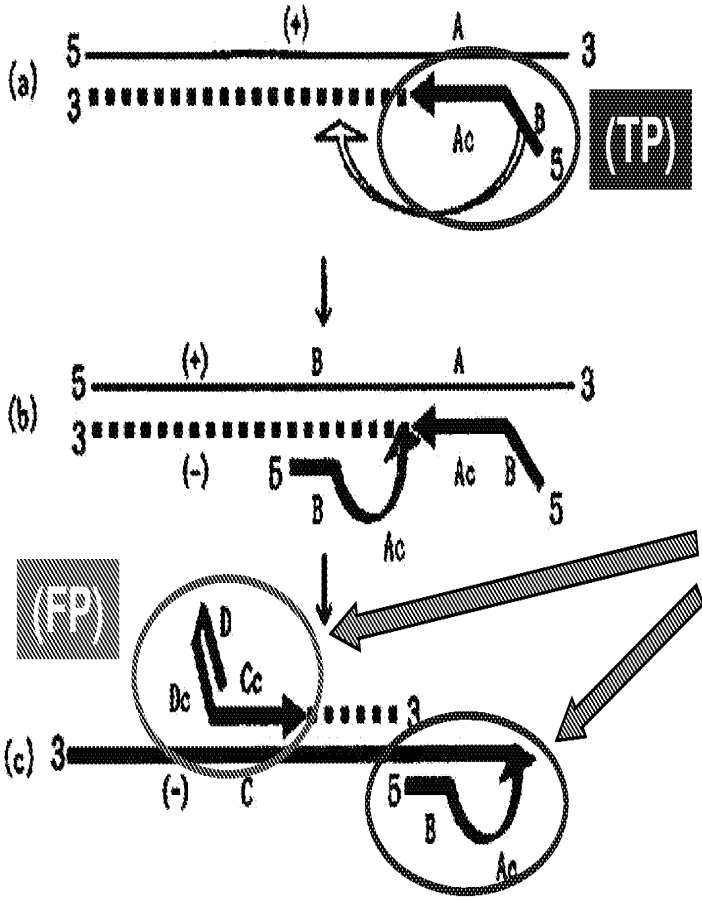
"Second primer" in Rabbani [0043] is NOT reverse (antisense) primer.

"this second segment is (iv) capable of providing for subsequent binding of a first segment of a **second primer** or nucleic acid construct **to the first portion of the specific nucleic acid sequence** under isostatic or limited cycling conditions. In so doing, a second primer extension is produced and that displaces a first primer extension."
(Rabbani p8 l37~39 [0042])

"second primer" in Rabbani [0043] is NOT reverse (antisense) primer (different from the "second primer" in the present invention).



FP quite different from the stem-loop structure of elongation of TP (1).

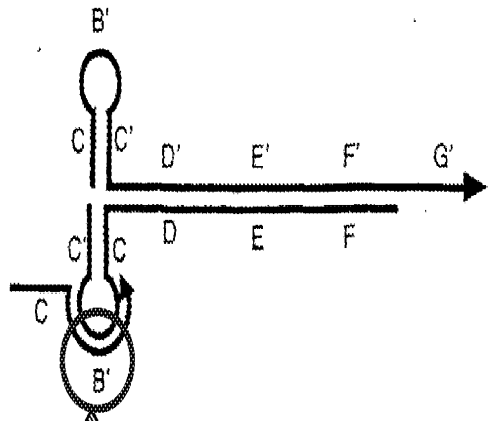


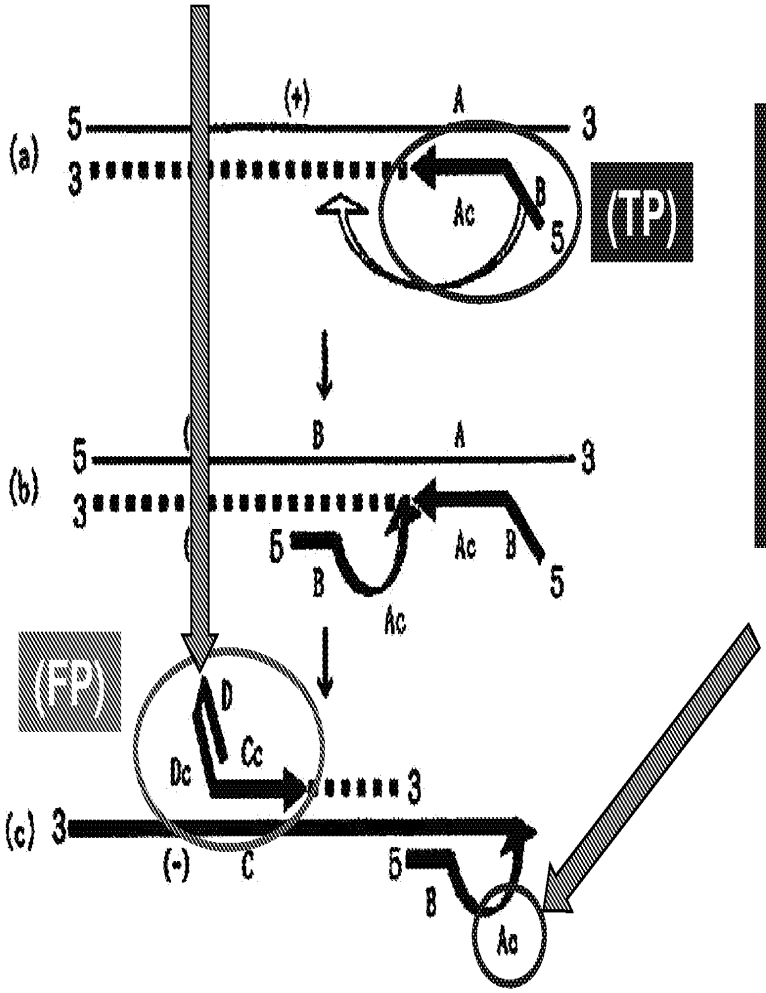
FP different in shape and function to the stem-loop structure in elongation of TP.

Stem-loop structure in elongation of TP

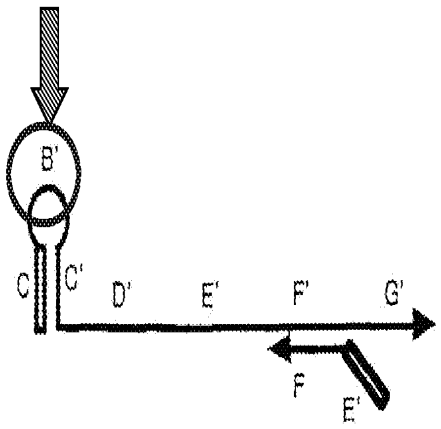
FP quite different from the stem-loop structure of elongation of TP (2).

FP does NOT have a sequence to which new primer hybridizes.

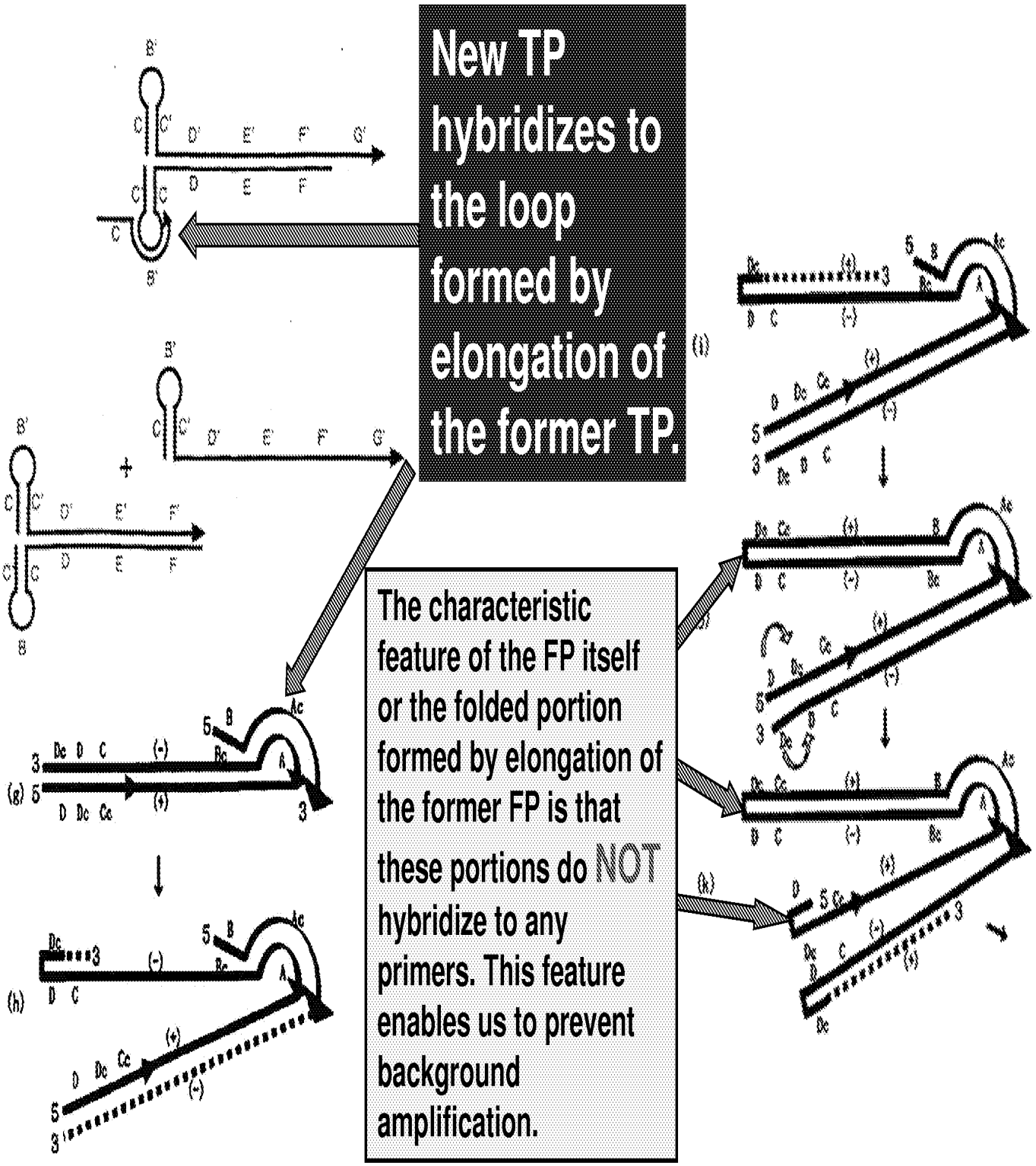




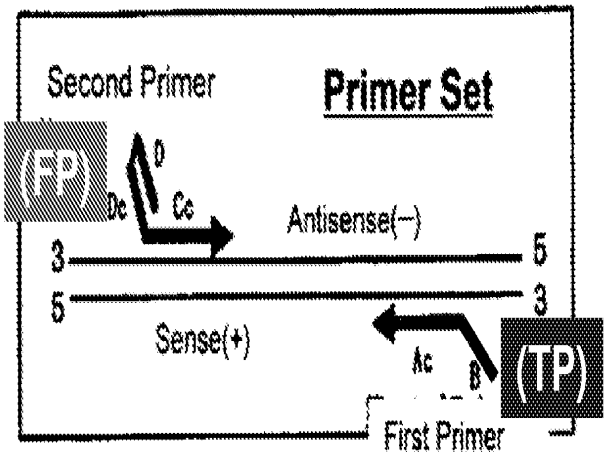
Stem-loop structure in elongation of TP has a sequence to which new TP hybridizes (FIGURE 2 ④ and FIGURE 4 ① in Rabbani, Fig. 3a (c) in the present application).

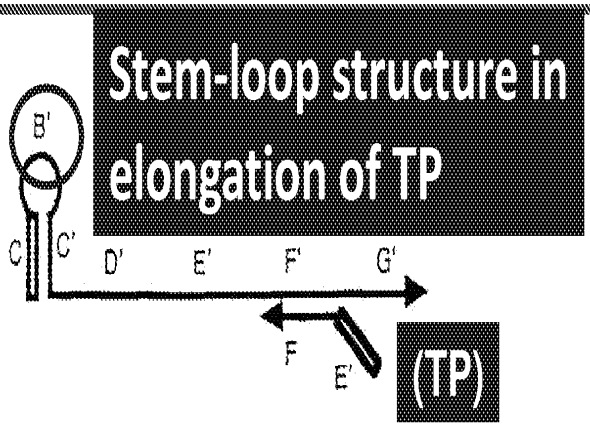


FP quite different from the stem-loop structure of elongation of TP (3).



Conclusions





(1) Stem-loop structure in elongation of TP (FIGURE 4 ① in Rabbani) is different in shape and function to FP.

(2) "Second primer" in Rabbani [0043] is a subsequent which hybridizes to the same portion to the initial primer, and NOT reverse (antisense) primer.

(3) So, a person of ordinary skill in the art is NOT motivated to reach the present invention from Rabbani and David.

Unexpectable advantages of the present invention by using TP and FP .

(1) Isothermal amplification

- The amplification occurs without thermal denaturation.

(2) Specific amplification

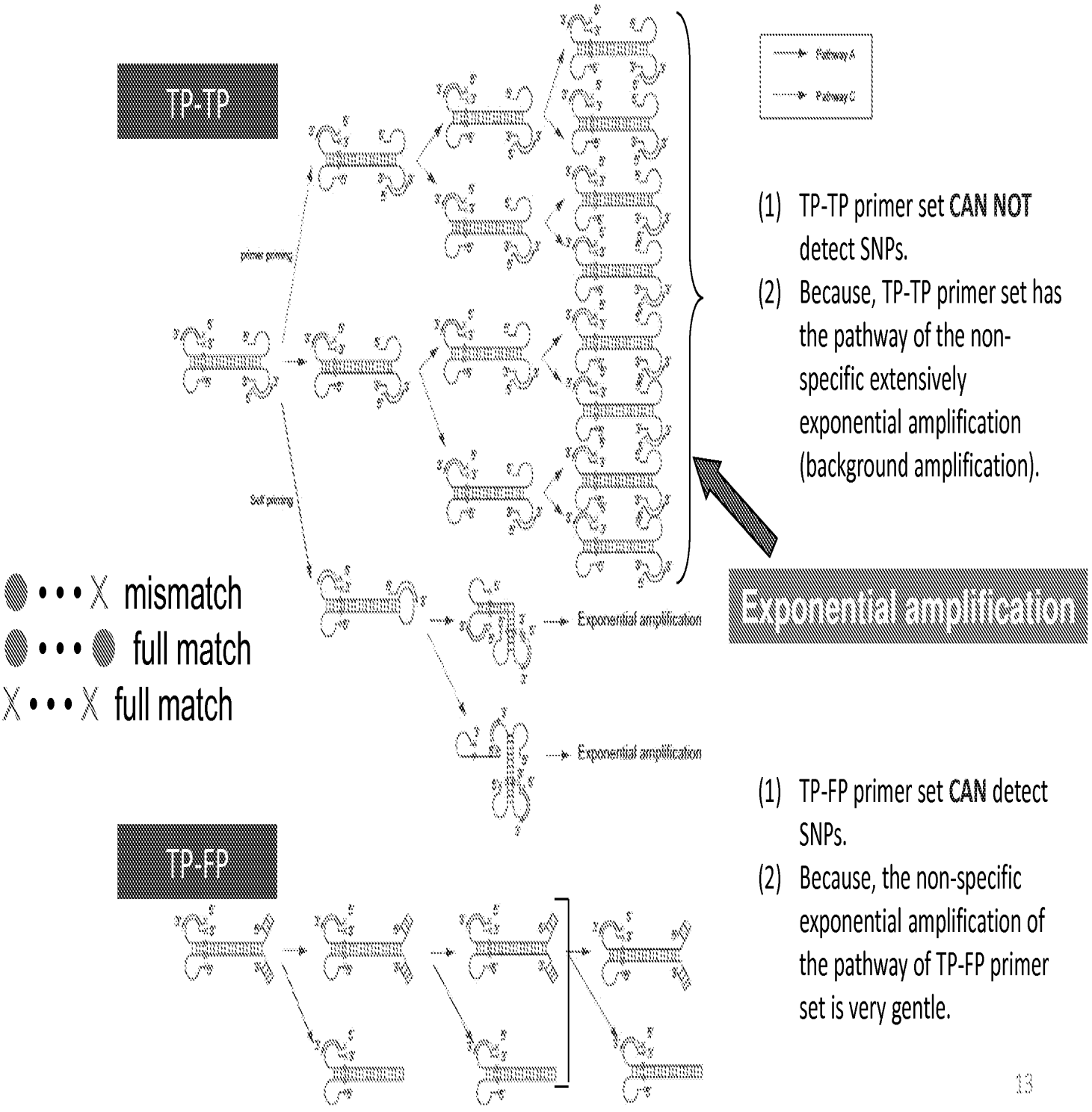
- The present invention can detect SNPs without non-specific amplification.

(3) Short time amplification

(4) Easy primer design

Mechanism of specific amplification (advantage (2))

The non-specific amplification **DOES NOT** occur in the present invention (TP-FP).



(1) TP-TP primer set **CAN NOT** detect SNPs.

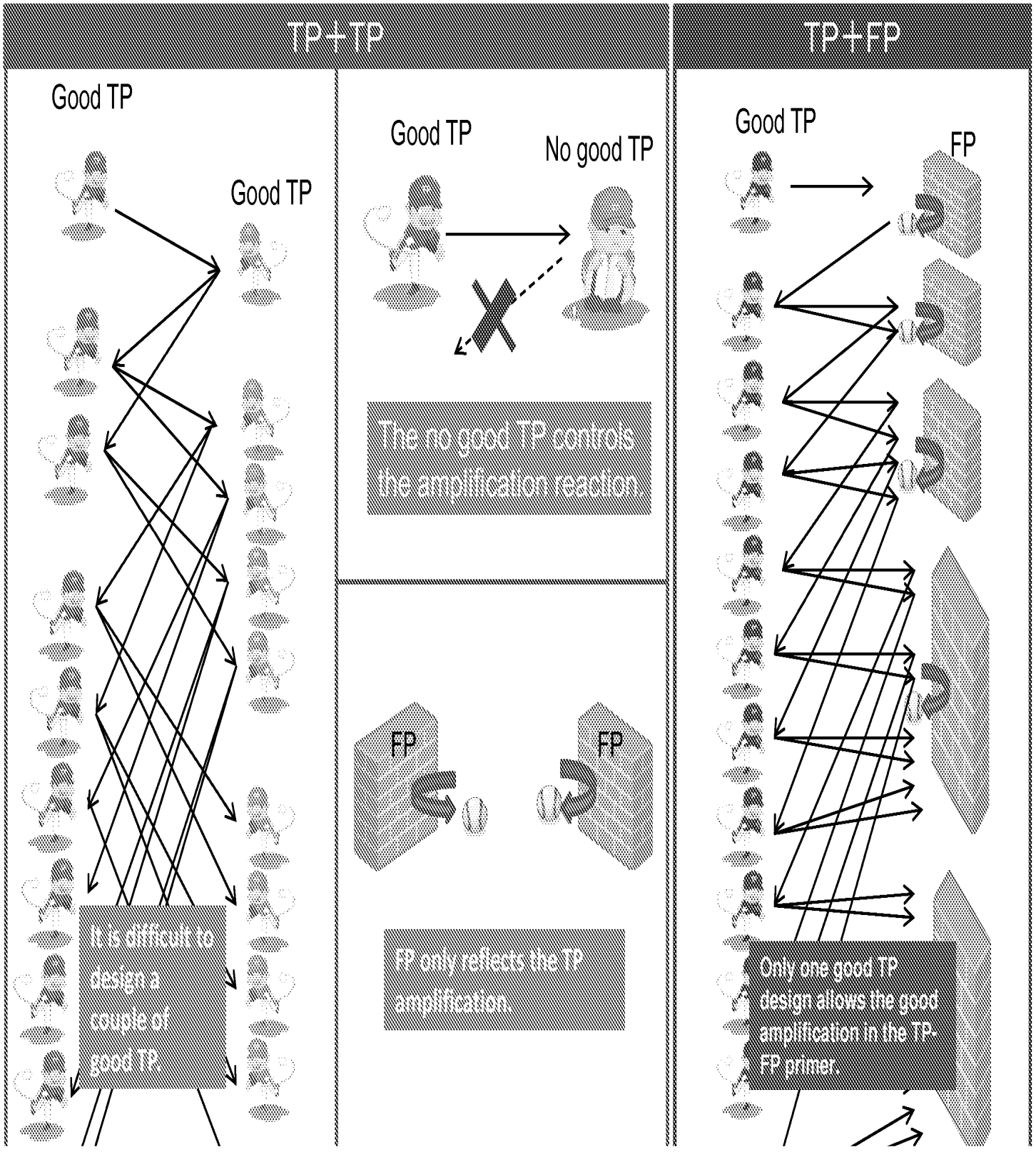
(2) Because, TP-TP primer set has the pathway of the non-specific extensively exponential amplification (background amplification).

Exponential amplification

(1) TP-FP primer set **CAN** detect SNPs.

(2) Because, the non-specific exponential amplification of the pathway of TP-FP primer set is very gentle.

Short time amplification (advantage (3))



Easy primer design(advantage (4))

(1) TP

(i) TP can amplify exponentially.

(ii) TP has a strong engine of amplification.

(iii) TP has two areas based on template sequence.

(2) FP

(i) FP can not amplify exponentially, but amplifies linearly.

(ii) FP is like a mirror which reflects TP amplification.

(iii) FP needs only one area based on template sequence.

(3) TP-TP primer set

(i) TP-TP primer set needs four areas based on the template sequence.

(ii) TP-TP Primer set needs the design of a couple of good TPs because the reaction is totally controlled by the presence of no good TP.

(iii) TP-TP Primer set is difficult to design.

(4) TP-FP primer set

(i) TP-FP primer set needs only three areas based on the template sequence. Thus, the distance (the number of bases) between TP and FP in TP-FP primer set can be made shorter than that between TP and TP in TP-TP primer set.

(ii) TP-FP Primer set needs the design of only one good TP because FP whose folded sequence can be designed in advance independently from template sequence **DOES NOT** control the reaction.

(iii) TP-FP Primer set is easy to design.